Perceptual and Motor Skills, 1982, 55, 515-519. © Perceptual and Motor Skills 1982

INTERACTIONS OF 2-PPM LEAD IN THE WATER SUPPLY WITH FOOD DEPRIVATION UPON MAZE-SWIMMING BEHAVIOR OF MICE[1]

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Summary.—Mice that had been given either tap water or 2 ppm lead in their drinking water and either severely food deprived (3 days before testing) or allowed food *ad libitum* demonstrated significant interactions of lead treatment by day by food condition and lead by block. Although not statistically significant, the food deprived-lead treated mice displayed more errors and longer latencies than the *ad libitum*-water controls. The food deprived-water controls and *ad libitum*-lead-treated mice displayed intermediate values. The importance of using multivariate statistical techniques that can evaluate dynamic repeated behavioral measurements is emphasized.

Most studies concerned with the effects of lead upon behavior: (1) have involved very large (and unrealistic) concentrations of lead, (2) have been plagued by the potentially confounding effects of body weight loss in treated animals, (3) have used static (single-measure) techniques, or (4) have included "control" subjects maintained on distilled water (Ogilvie, 1977; Quarterman, Morrison, & Humphries, 1976). However, significant alterations in maze-swimming behavior have been reported recently in mice treated with only 20 ppm of lead (McLean, Parker, & Persinger, 1982). Many of those effects were only evident because the analyses involved repeated-session and repeated within-session measures.

The present study was designed to determine the effects, if any, of still lower concentrations of lead (2 ppm) in the water supply. The possibility that the effects of the very low concentration of lead could be exacerbated by some second common environmental variable, in this case acute food deprivation, was also investigated.

METHOD

Subjects

Sixteen male and 16 female 60- to 90-day-old Wistar albino mice were used as subjects. Half the numbers of each sex had been reared in the Laurentian facility while the others had been obtained at 60 days of age from Canadian Breeding Farms.

Procedure

After 2 wk. of habituation to the colony conditions (see McLean, Parker,

[1]Supported in part by Canadian NSERC Grant No. A0445.
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& Persinger, 1982), animals were assigned to one of two lead treatments (2 ppm Pb or tap water). Ten days later, half of the numbers of mice in each treatment were assigned to one of two food conditions, *ad libitum* and 23-hr. food deprivation. The experiment was completed during January 1981 in four replicates with 1 wk. between replicates. Animals were housed singly during the entire experiment. Two males in the food-deprived groups (one lead, one tap water) died during the experiment.

Three days later, all subjects in a replicate began the three consecutive days of testing (9 trials/day) in the swimming maze following the procedure of McLean, Parker, and Persinger (1982). Body weights were taken each week and at the beginning of each test day. Animals were not tested under blind conditions. Four- and five-way analyses of variance with two factors repeated were completed using BMD software for the mean escape latencies (absolute, truncated and square root) and total errors (absolute and square root) per block.

RESULTS

The means and standard deviations for the mean truncated and square root (in parenthesis) values of the latencies (in sec.) and for the absolute

TABLE 1

MEANS AND STANDARD DEVIATIONS (±) FOR MEAN (IN SECONDS) TRUNCATED ESCAPE LATENCIES PER BLOCK AND TOTAL ERRORS PER BLOCK FOR EXPERIMENT II

		Lead Treatments	
		Tap Water ($n = 15$)	2 ppm Pb ($n = 15$)
Latency		43 ±30 (7.9±5.1)	62 ±40 (10.7±6.3)
Errors		1.8± 1.3 (1.3±0.5)	2.9± 2.3 (1.6±0.7)
		Food Condition	
		Ad Libitum ($n = 16$)	Deprived ($n = 14$)
Latency		43 ±34 (7.7±5.0)	64 ±38 (11.1±6.4)
Errors		1.9± 1.6 (1.3±0.5)	2.9± 2.0 (1.6±0.6)
		Gender	
		Female ($n = 16$)	Male ($n = 14$)
Latency		50 ±30 (9.1±5.0)	56 ±42 (9.6±6.9)
Errors		2.4± 2.0 (1.5±0.6)	2.3± 1.8 (1.4±0.6)
		Days	
	1 ($n = 31$)	2 ($n = 31$)	3 ($n = 31$)
Latency	31 ±32 (6.0±4.6)	58 ±46 (10.3±7.4)	72 ±50 (12.4±8.5)
Errors	2.0± 1.9 (1.3±0.6)	2.2± 2.4 (1.3±0.8)	2.9± 2.8 (1.4±0.9)
		Blocks	
	1 ($n = 31$)	2 ($n = 31$)	3 ($n = 31$)
Latency	46 ±33 (7.8±5.0)	34 ±41 (6.1±5.4)	60 ±41 (11.0±7.2)
Errors	2.9± 2.1 (1.6±0.6)	2.0± 2.2 (1.2±0.8)	2.2± 2.1 (1.3±0.7)

Note.—Means and standard deviations for the square roots of the absolute latencies and of the total errors are shown in parentheses.

and square root values of the total errors/block are shown in Table 1 for the three main effects (lead, food, and gender) and the two repeated measures. Since there were no major sex differences, gender was collapsed for subsequent analyses. There were no statistically significant main effects for the first measures although the lead-treated animals did have longer latencies than the controls ($Fs = 3.27$, (3.17), $df = 1/22$, $p < .10$). However, there were significant day ($Fs = 13.25$, (13.64), $df = 2/52$) and block ($Fs = 5.97$, (12.19), $df = 2/52$) effects. The only significant ($p < .05$) interaction, lead treatment by day by food condition ($Fs = 3.58$, (2.42), $df = 2/52$), is shown in Fig. 1. Covariance for body weight and *post hoc* analyses with replication series did not appreciably alter the results.

Although the lead-treated mice emitted more errors than the tap-water controls ($Fs = 3.03$, (2.35), $df = 1/26$) and the food-deprived mice emitted more errors than the *ad libitum* group ($Fs = 2.16$, (2.47), $df = 1/26$),

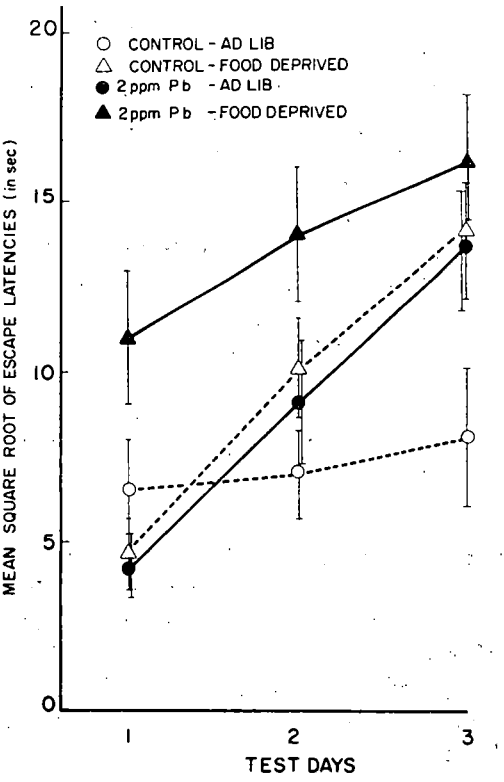

FIG. 1. Mean square root of the escape latencies (in sec.) for the different lead treatments (2 ppm Pb versus controls) and food condition (*ad libitum* versus 23-hr. food deprivation) over the three test days. Vertical bars indicate ±1 *SEM*.

the differences were not statistically significant. The means and *SD*s of absolute errors/block for the tap-water-*ad libitum*, tap-water-food-deprived, lead-*ad libitum*, and lead-food-deprived groups were 1.4 ± 0.9, 2.3 ± 1.5, 2.5 ± 2.6, 3.4 ± 2.5, respectively.

However, a significant interaction ($p < .05$) of lead by block ($Fs = 2.49, (5.74), df = 2/52$) was evident. Over the three test days, the lead-treated mice displayed more errors (especially during the third, final block) while the controls displayed fewer errors (especially during the final block). The effect was not altered when the data were covaried for body weights. A significant ($p < .01$) effect for block ($Fs = 3.49, (12.44), df = 2/52$) and an interaction of day by block ($Fs = 3.05, (3.92), df = 4/104$) were evident. No other interactions were statistically significant.

Three-way analysis of variance with one factor repeated (lead by food condition by days) indicated that the food regime was successful. A significant interaction of day by food deprivation ($F = 130.80, df = 2/52, p < .001$) was evident. The means and deviations for body weights on the day before the lead treatments, after 1 wk. of the treatments and on the final test day (six days of food deprivation) were 32.7 ± 3.2, 33.8 ± 3.0, and 33.6 ± 3.7 gm. for the *ad libitum* groups and 32.9 ± 4.0, 33.1 ± 4.0 and 25.0 ± 3.5 gm., respectively, for the 23-hr. food-deprived groups. However, there were neither significant effects for lead nor food condition and interactions of day by lead for body weights.

As an extra check for possible changes in body weight during the three consecutive test days, three-way analyses of variance with one factor repeated (across the three test days) were completed. During these three days, the food-deprived mice weighed (26.0 ± 3.5 gm) significantly ($p < .01$) less ($F = 16.97, df = 1/26$) than the *ad libitum* groups (33.5 ± 4.0 gm.). Again a significant ($p < .01$) effect for day ($F = 34.76, df = 2/52$) and interactions of day by food condition ($F = 22.93, df = 2/52$) existed. However, there was no evidence of any main effects of lead or interactions.

Discussion

The present study again indicates that experiments designed to evaluate statistically dynamic processes (repeated measures over time) can detect behavioral consequences of drinking low-level lead concentrations. The significant behavioral effects of lead exposure were evident in the interactions of day by lead, block by lead, or day by block by lead. These effects were obtained *without* the confounding loss of body weight known to be associated with higher lead concentrations.

The low levels of lead (2 ppm) required to produce significant behavioral changes are in stark contrast to the high concentrations reported in previous studies. For example, Ogilvie (1977) used 1% lead acetate (0.03 M)

or about 6300 ppm of Pb. However, he tested his animals on one day only and did not apply repeated-measure statistics. In addition, his mixtures required the use of double-distilled drinking water. The potentially adverse effect of electrolyte loss in the controls of Ogilvie's study was not checked by the use of tap-water treatments.

This experiment also demonstrated that the behavioral consequences of 2 ppm lead can be exacerbated when it is combined with another environmental manipulation. The lead plus food-deprivation group demonstrated twice the latencies as the food-deprivation group that received no lead or the lead group that was not food-deprived. These two groups averaged about twice the time of the tap water, *ad libitum* group. It is interesting that 2 ppm of lead in the water supply was associated with differences in latency similar to the more blatant assaults (75% free feed weight within six days) of 23-hr. food deprivation.

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Accepted August 6, 1982.